kβ3-kβ7 correspond to the five-stranded β-sheet found in the protein kinases. The kβ3-kβ4 loop corresponds to the protein kinase β1-β2 loop (also known as the glycine-rich or P-loop). This loop interacts closely with the phosphates of the bound ATP, but unlike the protein kinases, it contains no glycine. Instead, the side chain of Ser 806, a residue that is conserved in all PI3Ks, interacts with the β-phosphate (Fig. 2). Residue Lys 833 at the end of kβ5, corresponding to Lys 72 of c-AMP-dependent protein kinase, interacts with the α-phosphate of ATP. This residue is conserved in all PI3Ks and is covalently modified by Wortmannin (9). There are two metal binding sites (Fig. 2). Me I interacts with the conserved Asn 951 while Me II interacts with Asp 836 and Asp 964.

The link between the N- and C-terminal lobes is *via* a loop between strands kβ7 and kβ8. This loop forms the deepest wall of the ATP binding pocket and provides two hydrophobic contacts with the adenine moiety of the ATP. The C-terminal lobe forms a portion of the ATP binding site as well as the binding site for phospholipid substrates. The region between kα6 and kβ9 (residues 943-951) corresponds to the catalytic loop of the protein kinases. Mutations of residues in this loop analogous to Asp 946, Arg 947, Asp 950 and Asn 951 abolish kinase activity of PI3Ks (7,8).

The C-terminal lobe contains a segment (964-988) analogous to the activation loop in the protein kinases; this loop is essential for the substrate specificity of the PI3Ks (10). In the ATP/Lu^{3+} complex, much of this loop (968-982) is disordered. In the structure of an enzyme/chloramine T complex, all but two residues (Phe 975 and Leu 976) of this segment are visible, although high B-factors suggest that this loop is flexible. The activation loop is on the surface of the enzyme between the C-terminal

helix kα12 on one side and kα10 on the other. We have attempted to soak phospholipid analogues into PI3Kγ crystals, but no substrate was evident in the electron density. Consequently, we have modelled phospholipid headgroup binding, but because conformational changes are likely to occur in the activation loop and possibly in the C-terminal helix upon substrate binding, the model is only approximate. In this model, the headgroup is positioned in a cavity lined by the C-terminal helix kα12, the activation loop and the catalytic loop (Fig. 4). This would place the 5-phosphate of a PtdIns(4,5)P_2 adjacent to Lys 973 and the 1-phosphate near Lys 807 and Lys 808. The involvement of Lys 973 as a ligand of the 5-phosphate might explain why this residue is not present in the class II PI3Ks which do not phosphorylate phosphoinositides with a 5-phosphate. The basic residues nearest the 4-phosphate are Arg 947 and Lys 973. The specificity of the class III PI3Ks for phosphatidylinositol might be explained by their shorter activation loop that might not leave sufficient space to accommodate a 4-phosphate at the bottom of the headgroup-binding pocket. PI3Kδ autophosphorylates in a region just beyond the C-terminal helix kα12 (11), resulting in enzyme inhibition probably by sterically preventing substrate binding. The proximity of the C-terminal segment to the substrate binding site is consistent with autophosphorylation of this region.

The mechanism originally proposed for the enzymatic activity of protein kinases involved participation of a residue acting as a general base to deprotonate the hydroxyl of the substrate generating a nucleophile that would attack the γ-phosphate of ATP. In cAMP-dependent protein kinase (cAPK), Asp 166 has been proposed to play the role of this general base. This residue corresponds to Asp 946 of the PI3Kγ 946-DRH-948 sequence that is conserved in all PI3Ks. However, in the structure of

PI3Kγ, Asp 946 is not positioned so that it could have the role of a general base catalyst. The constellation of residues in the active site in the presence of ATP/metal suggests that Asp 946 may simply have a structural role in maintaining the integrity of the ATP-binding pocket. Therefore, either the enzyme has no general base catalyst, in which case the mechanism could be primarily dissociative, involving a metaphosphate transition state (12) or a different residue assumes this role in the PI3Ks. One candidate for the role of a general base may be His 948. Although the side-chain of His 948 is not near the γ-phosphate of ATP, a rotation around χ1 would place the side chain in a location such that it might interact with the 3-hydroxyl of the lipid headgroup.

PI3Ks have been identified as one of the effectors for Ras proteins (reviewed in (13)). Binding of PI3K to Ras is affected by mutations in both switch I and switch II regions of Ras (residues 30-38 and 60-76, respectively) (14,15). These two regions are known to change conformation upon GTP binding and serve as binding sites for a diverse array of downstream effectors. However, mutations in these switch regions have been identified that differentially affect binding of various effectors.

The structure reveals that the RBD of PI3Kγ (residues 220-311) has the same fold as the RBD of Raf (16) and RalGDS (17), two other well-characterised effectors of Ras (Fig 5). The RBD of PI3K consists of a five-stranded mixed β-sheet (Rβ1-Rβ5) flanked by two α-helices (Rα1 and Rα2). Residues 228-230 (in the Rβ1/Rβ2 loop) and 257-265 (in the Rα1/Rβ3 loop) are disordered.

The crystal structure of Ras-related protein Rap1A in complex with the RBD of protein kinase c-Raf (16) and the structure of Ras in complex with the RBD of RalGDS (17), suggests a structural basis for effector specificity. For both of these